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INVITED REVIEW

Long noncoding RNAs in prostate cancer: overview and clinical implications

Bhavna Malik¹, Felix Y Feng^{2,3}

Prostate cancer is the second most common cause of cancer mortality among men in the United States. While many prostate cancers are indolent, an important subset of patients experiences disease recurrence after conventional therapy and progresses to castration-resistant prostate cancer (CRPC), which is currently incurable. Thus, there is a critical need to identify biomarkers that will distinguish indolent from aggressive disease, as well as novel therapeutic targets for the prevention or treatment of CRPC. In recent years, long noncoding RNAs (lncRNAs) have emerged as an important class of biological molecules. lncRNAs are polyadenylated RNA species that share many similarities with protein-coding genes despite the fact that they are noncoding (not translated into proteins). They are usually transcribed by RNA polymerase II and exhibit the same epigenetic signatures as protein-coding genes. lncRNAs have also been implicated in the development and progression of variety of cancers, including prostate cancer. While a large number of lncRNAs exhibit tissue- and cancer-specific expression, their utility as diagnostic and prognostic biomarkers is just starting to be explored. In this review, we highlight recent findings on the functional role and molecular mechanisms of lncRNAs in the progression of prostate cancer and evaluate their use as potential biomarkers and therapeutic targets.

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Keywords: biomarker; long noncoding RNAs (lncRNAs); prostate cancer

INTRODUCTION

In the past decade, numerous studies have helped unravel the molecular and biological processes that contribute to prostate cancer (PCa) development. With the advent of whole genome- and exome-sequencing, scientists have deciphered various genomic alterations contributing to PCa pathogenesis.^{1,2} The loss of one copy of the tumor suppressor *PTEN* has been found in approximately 60% of men with PCa.³ Mutations in *p53*, *BRCA1* and *BRCA2*, and loss of *RB* have also been reported in smaller proportions of PCa cases.^{4–6} Moreover, chromosomal rearrangements such as *TMPRSS2-ETS* gene family fusions have been found frequently in Caucasian PCa cohorts.⁷ In addition to mutations and chromosomal translocations, epigenetic alterations have also been associated with PCa. For instance, hypermethylation at the promoter regions of *PTEN*, *RB*, and *CDH1* is associated with advanced PCa.⁸

However, while the majority of these previous studies has focused on protein-coding genes, recent studies have suggested that only 2% of the genome is comprised of protein-coding genes.⁹ Strikingly, the vast majority of the genome (around 70%) is actively transcribed, meaning that the majority of the human transcriptome is comprised of noncoding RNAs (ncRNAs), genes that are transcribed into RNA but not translated into protein.⁹ ncRNAs are classified by their size as small ncRNAs (<200 bp) or long ncRNAs (>200 bp).¹⁰ One particular class of small ncRNAs, microRNAs (miRNAs), has been extensively studied in the literature. MiRNAs negatively regulate the protein expression of a gene via binding to the 3' untranslated region of the target gene

mRNA.¹¹ As opposed to miRNAs, long noncoding RNAs (lncRNAs) are much less studied. lncRNAs are further categorized as intergenic, intronic, exonic, antisense, or overlapping based on the genomic location relative to a protein-coding gene, as shown in **Figure 1**.^{12,13} The significance of lncRNAs in cancers is rapidly gaining attention because of recent studies discovering tens of thousands of novel, unannotated lncRNAs.^{14,15}

In the past, a major hurdle in lncRNA research was the inability of conventionally utilized microarrays to detect lncRNA expression due to the lack of lncRNA-directed probes, hence limiting our understanding of the role of lncRNAs in prostate cancer. However, recent advances in transcriptome sequencing (RNASeq) technologies have allowed the study of gene expression in an unbiased manner, resulting in the discovery of thousands of novel RNA species including lncRNAs. One initial study identified 121 lncRNAs, termed as PCATs (prostate cancer-associated noncoding transcripts), using *ab initio* computational approaches on RNASeq data from 102 prostate cancer tissue samples.¹⁵ The expression pattern of these 121 lncRNAs distinguished benign, localized, and metastatic prostate samples.¹⁵ More recently, a significant effort has been made both by our group and others to discover a landscape of lncRNAs in the human transcriptome using bioinformatics-based approaches. This study employed RNAseq data from 25 independent studies comprising over 7000 RNAseq libraries from tumors, normal tissues, and cell lines.¹⁴ Over 50 000 lncRNAs were identified, of which 79% were novel or unannotated, thus quadrupling the number of known lncRNAs.¹⁴ Importantly, about 8000 lncRNAs

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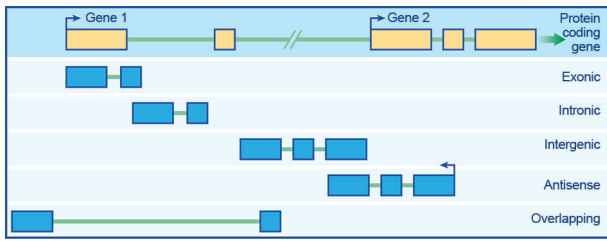


Figure 1: Classification of long noncoding RNAs. Long noncoding RNAs (lncRNAs) are categorized as exonic, intronic, intergenic, antisense, or overlapping based on their genomic location relative to a protein-coding gene. Exonic lncRNAs share exons with a protein-coding gene. Intronic lncRNAs are transcribed within the introns of a protein-coding gene. Intergenic lncRNAs are transcribed within the regions between two protein-coding genes. Antisense lncRNAs are located on the opposite strand from a protein-coding gene. Overlapping lncRNAs are transcripts that contain a protein-coding gene within its intron.

were characterized to be lineage- or cancer-specific, suggesting that lncRNAs are very attractive as potential biomarkers or therapeutic targets.

POTENTIAL MECHANISMS AND FUNCTIONS OF LNCRNAS

lncRNAs are nonprotein-coding genes characterized by several features. While the majority of lncRNAs is polyadenylated and transcribed by RNA polymerase II, a significant subset is nonpolyadenylated and transcribed by RNA polymerase III. As other transcribed genes, lncRNAs harbor epigenetic marks, such as trimethylation of histone 3 lysine 4 (H3K4me3) at the promoter region and trimethylation of histone 3 lysine 36 (H3K36me3) throughout the body of the gene.¹⁶ Moreover, lncRNAs exhibit frequent splicing of multiple exons and are expressed in a cell- and tissue-specific manner.^{9,17,18}

Similar to protein-coding genes, lncRNAs vary considerably in function. The function of lncRNAs often relates to the transcriptional regulation of genes leading to differential mRNA processing. There are different ways by which lncRNAs function to regulate target gene expression, as shown in **Figure 2**. The most common mode of gene regulation involves an epigenetic mechanism that typically results in transcriptional repression by coupling with chromatin-remodeling or histone-modifying protein complexes.¹⁹ Among all the chromatin remodeling complexes, the most common protein partners for lncRNAs are Polycomb Repressive Complexes 1 and 2 (PRC1 and PRC2). lncRNAs serve as scaffolds that mediate the recruitment of these Polycomb Repressive Complexes to certain genomic regions to guide transcriptional regulation.

In addition to transcriptional regulation by epigenetic changes, lncRNAs are also known to be involved in mRNA processing, including mRNA stability, splicing, and translation (**Figure 2**). These posttranscriptional RNA modifications, including alternative splicing, involve the assembly of RNA-processing factors containing nuclear domains at certain genomic sites.²⁰ Moreover, lncRNAs can function as decoys or molecular sponges for miRNAs that target protein-coding mRNAs. In this way, lncRNAs sequester miRNAs to regulate gene expression indirectly.²¹ Furthermore, emerging evidence suggests a role for certain lncRNAs, termed enhancer RNAs (eRNAs), in gene regulation via influencing the activity of gene enhancers. These eRNAs are transcribed from gene enhancers, and can cooperate with lineage-specific complexes, such as FOXA1 and AR, to facilitate hormone signaling pathways.²²

Mechanistically, lncRNAs can be characterized as cis- and trans-regulators of gene expression based on whether they target genes

that are local or distant, respectively, from their genomic location. For example, lncRNAs have been shown to regulate gene expression in both cis- and trans-based approaches by facilitating the recruitment of PRC2 complexes to local and distant genes.^{23,24} Taken together, it is clear that the mechanisms by which lncRNAs regulate gene expression are quite complex, with further investigation necessary to more clearly decipher the role of lncRNAs.

Through the functions highlighted in **Figure 2**, lncRNAs can function as oncogenes or tumor suppressors by modulating physiological and pathological processes, including cell growth and differentiation, stem cell reprogramming, and disease progression. Many lncRNAs have been shown to be either up- or down-regulated in various cancers, including prostate cancer, and are associated with disease progression. In fact, using high-throughput approaches to interrogate RNA expression in over a thousand prostate cancer patients treated with prostatectomy, a recent study from our group demonstrated that among all protein-coding genes and lncRNAs annotated at the time of the study, the lncRNA *SChLAP1* was the top overexpressed gene in cancers that subsequently metastasized versus those that did not.²⁵ The finding that the prognostic value of lncRNAs may rival or outperform that of top protein-coding genes has significant implications for clinical biomarker development in prostate cancer. Below, we highlight several lncRNAs that have been implicated in prostate carcinogenesis or progression.

PROSTATE CANCER-ASSOCIATED LNCRNAS

Since the initial discovery of lncRNAs such as *XIST* and *H19*, there have been dramatic advances in the high-throughput technologies, thereby enabling the discovery of RNA transcripts in an unbiased manner.^{15,26–31} Since then, many lncRNAs have been linked to tumorigenesis, either as oncogenes or tumor suppressors. While the underlying mechanism of many of these lncRNAs remains to be elucidated, it is clear that lncRNAs contribute to dysregulation of gene expression in prostate cancer, which then results in cancer initiation, development, and progression.¹⁵

One of the first lncRNAs discovered to be highly upregulated in prostate cancer (PCa) was Prostate Cancer Antigen 3 (*PCA3*), which was initially discovered via expression profiling of PCa samples.³² *PCA3* was shown to be significantly overexpressed in PCa versus adjacent noncancerous prostate tissues in 95% of radical prostatectomy specimens.³² Extensive analysis of the genomic loci of *PCA3* (Chr9q21–22) demonstrated no open reading frame for this gene, consistent with a noncoding RNA transcript.³² A preclinical study suggested that knockdown of *PCA3* hinders PCa cell viability and alters the expression of AR target genes.³³ More recently, it was reported that *PCA3* is antisense to the tumor-suppressive protein-coding gene *PRUNE2* and downregulates the expression of *PRUNE2* via RNA editing mediated by a supramolecular complex containing adenosine deaminase acting on RNA (ADAR) family members.³⁴ Following the discovery of *PCA3*, other lncRNAs including Prostate Cancer-associated ncRNA Transcript 1 (*PCAT1*) and Second Chromosome Locus Associated with Prostate-1 (*SChLAP1*) were found to be differentially expressed in prostate cancer versus nonneoplastic prostate tissues.^{15,35}

PCAT1 was discovered as a prostate cancer-associated intergenic ncRNA in a cohort of 102 prostate tissues and cell lines via high-throughput RNAseq studies.¹⁵ *PCAT1* is highly prostate-specific and is remarkably upregulated in a subset of localized and metastatic prostate cancer tissues compared to adjacent nonneoplastic prostate tissues.¹⁵ The mechanisms by which *PCAT1* contributes to prostate

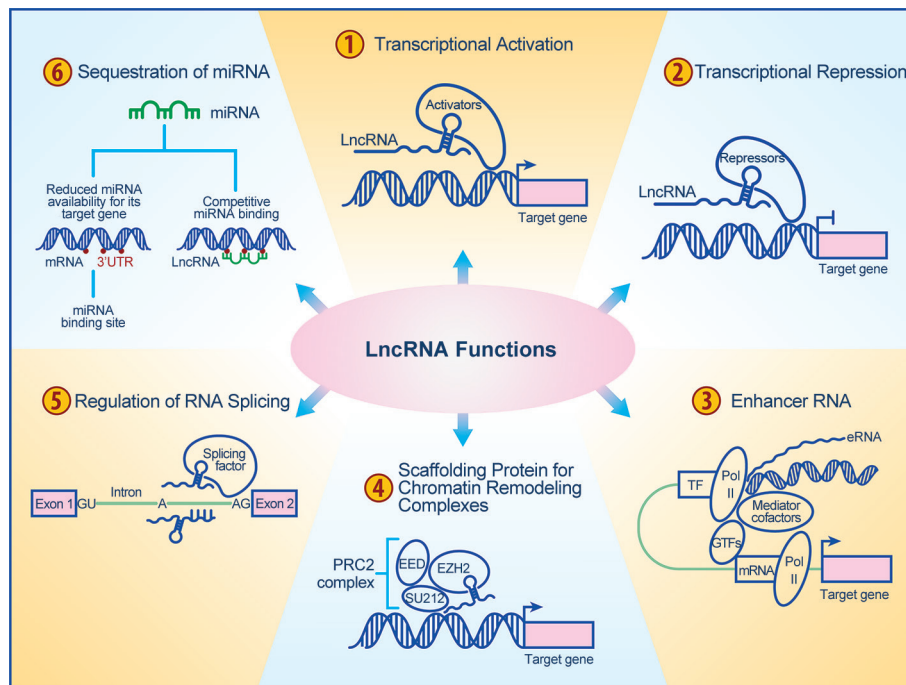


Figure 2: Functions of long noncoding RNAs. Long noncoding RNAs (lncRNAs) regulate target gene expression in a variety of approaches, including the following: (1) LncRNAs can interact with transcriptional activators, thereby leading to target gene activation. (2) LncRNAs may mediate transcriptional repression either by directly affecting tumor suppressor signaling or by acting as decoy to keep transcriptional activators away from chromatin. (3) LncRNAs transcribed from gene enhancers (eRNAs) may regulate signaling by recruiting lineage-specific complexes. (4) LncRNAs may serve as scaffolding proteins by recruiting chromatin remodeling complexes, including PRC1 and PRC2. (5) LncRNAs may regulate RNA splicing either by interacting with splicing factors or by binding the splicing junctions of pre-mRNA. (6) LncRNAs may serve as molecular sponges by harboring binding sites for miRNAs and titrating them away from their mRNA targets. eRNA: enhancer RNA, GTF: general transcription factors, LncRNA: long noncoding RNA, mRNA: messenger RNA, miRNA: microRNA, Pol II: RNA polymerase II, PRC2: polycomb repressive complex 2, TF: transcription factors, UTR: untranslated region.

carcinogenesis and progression have been studied in a series of preclinical studies.^{36,37} First, *PCAT1* represses *BRCA2*, a DNA repair gene critical to homologous recombination, by regulating its 3' untranslated region (UTR). This repression leads to a functional deficiency in homologous recombination, resulting in sensitivity to PARP1 inhibitors and radiation.³⁶ Second, *PCAT1* also promotes prostate cell proliferation through the upregulation of c-Myc.³⁷ Collectively, these data suggest that *PCAT1* contributes to disease progression via two complementary approaches: one where *PCAT1* represses *BRCA2* to create deficiencies in DNA damage repair to promote carcinogenesis and another where *PCAT1* facilitates prostate cell proliferation via regulating c-Myc. Given that DNA repair defects and unchecked proliferation represent two of the hallmarks of cancer, *PCAT1* may represent a prostate cancer biomarker that may hold value as both a biomarker prognostic of outcome but also predictive of response to particular therapies.

SchLAP1 was discovered as a highly expressed intergenic lncRNA associated with aggressive disease in subset of prostate cancer patients via cancer outlier profile analysis (COPA).³⁵ As described earlier, *SchLAP1* is notable for being one of the genes most enriched in expression in prostate cancers that will metastasize compared to those that do not.²⁵ *SchLAP1* expression has been shown to be an independent predictor of aggressive prostate cancer when accounting for standard clinicopathological factors, with high *SchLAP1* expression associated with biochemical recurrence, metastatic progression, and prostate cancer-specific mortality.²⁵ *SchLAP1* also significantly promotes cancer cell proliferation, invasion, and metastasis *in vitro* and *in vivo*. Mechanistically, *SchLAP1* facilitates aggressive phenotypes associated with cancer by antagonizing the tumor suppressive SWI/SNF

(Switch/Sucrose Nonfermenting) chromatin remodeling complex. The multiprotein SWI/SNF complex regulates gene transcription by physically moving nucleosomes at the gene promoters.³⁸ *SchLAP1* has been shown to interact with SNF5, a key component of SWI/SNF complex, and impairs the genomic binding of SNF5, thereby antagonizing the tumor suppressive function of SWI/SNF complex.³⁵ *SchLAP1* is currently developed as a prognostic biomarker using an RNA *in situ* hybridization assay.^{39,40}

Recent studies have also identified that lncRNAs may interact with the androgen receptor (AR), a well-known driver of prostate cancer. Two lncRNAs, *PCGEM1* and *PRNCR1*, have been proposed to be highly upregulated in primary prostate cancer versus normal prostate epithelium.^{41,42} In laboratory studies, increased cell proliferation and colony formation were observed with overexpression of *PCGEM1*, along with attenuated apoptotic response.⁴³ In addition, the knockdown of *PRNCR1* resulted in decreased cell viability.⁴⁴ These lncRNAs have been reported to bind AR and enhance AR-mediated gene activation programs.^{41,42} However, this area requires further study as the second study could not confirm the role of *PCGEM1* and *PRNCR1* in prostate cancer progression and AR signaling.⁴⁵ In addition, another recent publication demonstrated the upregulation of *PCGEM1* but not *PRNCR1* in AR+/androgen-dependent PCa xenograft models.⁴⁶ In addition to its potential AR-associated roles, *PCGEM1* may regulate tumor metabolism via c-Myc activation, by interacting physically with c-Myc and enhancing its chromatin recruitment and transactivation activity.⁴⁷

Several other lncRNAs have also been implicated as mediators or modulators of AR signaling. One study suggested that reciprocal regulation between the lncRNA *PlncRNA-1* and AR contributes

to oncogenic phenotypes *in vitro*.⁴⁸ Another androgen responsive lncRNA, C-terminal binding protein 1-antisense (*CTBP1-AS*), was demonstrated to promote both androgen-dependent and castration-resistant tumor growth by directly repressing the expression of its antisense gene *CTBP1*, a known AR corepressor.⁴⁹ More recently, a novel lncRNA cluster *DRAIC/PCAT29* has been shown to inhibit cancer cell migration and invasion.^{50,51} Mechanistically, the expression of *DRAIC* is repressed by binding of AR to the *DRAIC* locus but is induced by binding of FOXA1 and NKX3-1 to the same locus as AR. Together, these studies suggest that as the expressions of FOXA1 and NKX3-1 decrease with prostate cancer progression, there is decreased expression of the tumor suppressive *DRAIC/PCAT29* lncRNAs, leading to aggressive phenotypes.^{50,51}

Outside of AR, lncRNAs have been demonstrated to be involved in mediating the function of other potential prostate cancer drivers. The estrogen receptor alpha ($ER\alpha$) is expressed in subsets of PCa, independent of AR status, and may be associated with aggressive disease. Chakravarty *et al.* developed an $ER\alpha$ -specific noncoding transcriptome signature, and used this signature to identify Nuclear Enriched Abundant Transcript 1 (*NEAT1*) as the most significantly overexpressed $ER\alpha$ -regulated lncRNA in PCa.⁵² This group also demonstrated that PCa cells with high expression of *NEAT1* are resistant to androgen receptor antagonists.⁵² Another group identified 145 previously unannotated lncRNAs associated with castration-resistant prostate cancer (CRPC) and characterized one of these, *PCAT5*, as a regulatory target of the transcription factor ERG, which is activated in 50% of all prostate cancers.⁵³ Furthermore, by profiling androgen-dependent versus androgen-independent cell lines, another team recently identified *Linc00963* as a lncRNA which regulates the epidermal growth factor receptor signaling pathway to promote cell growth, migration, and invasion.⁵⁴

Another intriguing lncRNA in prostate cancer biology is Antisense Noncoding RNA in the *INK4* Locus (*ANRIL*), which has been shown to have an important role in cancer biology and is one of the best studied natural antisense transcript genes. It is an antisense transcript overlapping the tumor suppressor *INK4b-ARF-INK4a* gene cluster and is one of the most frequently altered lncRNAs in cancer.⁵⁵ There is either homozygous deletion or transcriptional silencing of the *ANRIL* gene cluster in almost 40% of human cancers.⁵⁶ The *INK4b-ARF-INK4a* gene cluster plays an important role in stress-induced apoptosis, cell cycle inhibition, and senescence, and the expression of this gene cluster has been shown to be repressed by the expression of *ANRIL*.^{55,57,58} The expression of *ANRIL* is higher in preneoplastic prostate epithelial tissues compared to untransformed prostate epithelial tissues. In addition, there are higher levels of *ANRIL* in prostate cancer relative to normal prostate epithelial cells with a corresponding decrease in the expression of *INK4a*.⁵⁹ In coordination with the PRC1 and PRC2 complexes, *ANRIL* leads to the transcriptional silencing of *INK4b-ARF-INK4a* locus via directly binding to *INK4b* transcripts. Moreover, in *ANRIL* knockdown studies, reduced levels of histone H3 lysine K27 methylation (H3K27me) has been reported at the *INK4b-ARF-INK4a* locus.⁵⁹ In addition, the role of *ANRIL* has also been studied in DNA damage response. Upon DNA damage, the expression of *ANRIL* is induced by E2F1 transcription factor in an ATM-dependent manner.⁶⁰ Further, the elevated *ANRIL* expression suppresses the expression of *INK4b-ARF-INK4a* locus. Thus, *ANRIL* could represent an interesting therapeutic target to sensitize cancers to DNA damaging drugs.

In prostate cancer, the lncRNA Metastasis-associated Lung Adenocarcinoma Transcript 1 (*MALAT1*) is involved in mRNA splicing

and is highly upregulated. *MALAT1* is an intergenic lncRNA on chromosome locus 11q13.1 that is thought to regulate gene expression through mRNA splicing and editing.⁶¹ *MALAT1* is primarily located in nuclear speckles and overexpressed in a variety of human cancers, including prostate cancer, and has been linked to poor prognosis.^{61–64} Moreover, knockdown of *MALAT1* in prostate cancer cell lines abrogates cell growth, migration and invasion, and induced G0/G1 cell cycle arrest. Therapeutically, *MALAT1* has been targeted in prostate cancer xenografts with intratumoral delivery of *MALAT1* siRNA, resulting in significant reduction in tumor growth and metastasis.⁶⁵

In contrast to the many oncogenic lncRNAs, fewer have been reported as tumor suppressor lncRNAs in prostate cancer. Growth Arrest-Specific Transcript 5 (*GAS5*) is a lncRNA that is highly upregulated in normal prostate epithelial cells but decreases in expression in prostate cancer cell lines. *GAS5* manifests multiple isoforms that constitute approximately 12 exons. Mechanistically, *GAS5* promotes cell apoptosis by antagonizing glucocorticoid receptor (GR) signaling axis in breast cancer. Similarly in prostate cancer, *GAS5* is suspected to regulate androgen receptor-mediated signaling to prevent the progression to metastatic castration-resistant disease.⁶⁶ Another lncRNA, Maternally Expressed Gene 3 (*MEG3*) has been shown to be downregulated in prostate cancer cell lines and primary tumors compared to normal tissues. *MEG3* is proposed to induce apoptosis in both p53 dependent and independent manners.^{67,68}

Our understanding of the potential roles of long noncoding RNAs in prostate cancer is starting to develop. However, given the discovery of >40 000 novel lncRNAs on recent transcriptome sequencing studies, it is also clear that much additional research needs to be performed in this area, to understand the molecular mechanisms underlying these genes. In addition, given that many of these lncRNAs are highly tissue- or lineage-specific, there is a clear need to pursue top candidates as potential biomarkers and therapeutic targets. In the following section, we discuss the potential clinical significance of lncRNAs in prostate cancer.

OPPORTUNITIES TO UTILIZE LNCRNAs IN THE CLINICAL MANAGEMENT OF PROSTATE CANCER

In the clinical management of prostate cancer, there is a critical need to better tailor therapy based on individual tumor characteristics. To improve the personalization of therapy for patients, two goals need to be achieved: (1) the identification of biomarkers to distinguish indolent from aggressive disease, in the context of diagnosis or work-up of localized disease and (2) the discovery of novel prostate cancer drivers, which can serve as new therapeutic targets in subsets of patients. Therefore, lncRNAs have the potential to contribute toward both of these goals.

As potential prostate cancer diagnostic and prognostic biomarkers, lncRNAs exhibit several ideal qualities. First, certain lncRNAs are expressed at extremely high levels in subsets of cancers and exhibit outlier profiles,¹⁴ which facilitates their detection in both tissue and bodily fluids. Second, significant subsets of lncRNAs are extremely specific for a particular cancer, considering that a recent study discovered approximately 8000 novel lncRNAs which are extremely cancer- or lineage-specific.^{14,25} A number of these lncRNAs are specific for prostate cancer,²⁵ and this specificity is an ideal trait for a potential noninvasive biomarker. Finally, lncRNAs represent a vastly unexplored area of cancer biology, and given that they outnumber protein-coding genes,¹⁴ there are likely many clinically relevant lncRNA biomarkers that are, to date, uncharacterized.

Up to now, the best-studied lncRNA biomarker is *PCA3*, which has been explored extensively as a urinary biomarker. Following the

initial discovery and characterization of *PCA3* as a highly overexpressed lncRNA specific to prostate cancer,³² a clinical assay was developed and introduced for the detection of urinary *PCA3* levels. This assay, named the ProgenSA *PCA3* assay,⁶⁹ required urine specimens to be obtained after digital rectal examination (3 strokes to each lobe), and quantified *PCA3* transcript expression based on transcription-mediated amplification and hybridization, and normalized *PCA3* levels based on prostate-specific antigen (*PSA*) transcript levels. Early studies demonstrated that the *PCA3* test improved the ability to diagnose prostate cancer, with a univariable AUC of 0.69 (compared to an AUC of 0.55 for *PSA*) that increased to 0.75 in a multivariable model with other clinical factors.⁷⁰ *PCA3* was demonstrated to be independent of *PSA* levels, prostate volume, or age.⁷¹ Based on these findings and others, the Food and Drug Administration approved the *PCA3* assay for use as a diagnostic test in men with a previous negative biopsy.^{72,73} Subsequent studies have focused on identifying the optimal cut-off score of *PCA3* in the context of clinical use. A meta-analysis from Luo *et al.* evaluated the performance of threshold scores ranging from 20 to 35 and concluded that a cut-off of 20 was superior to 35 in the repeat biopsy setting, with a sensitivity of 93% and a specificity of 64%.⁷⁴ In the context of the National Cancer Institute Early Detection Research Network validation trial, Wei *et al.* confirmed that *PCA3* scores <20 were associated with an extremely low rate of high-grade cancers on repeat biopsy.⁷⁵ In more recent years, studies have investigated the *PCA3* test in the setting of initial (rather than repeat) biopsy and have compared the *PCA3* to other assays. Overall, these studies have suggested that in limited cohorts, *PCA3* may not perform as well compared to other diagnostic tests, such as multi-parametric MRI or the Prostate Health Index test.⁷⁶ These results indicate that while *PCA3* may outperform *PSA*, further studies need to be performed to define the optimal clinical settings for its use.

While the *PCA3* assay is designed to diagnose prostate cancer, its utility as a prognostic biomarker is much more limited as it can detect both higher-grade and lower-grade disease.⁷⁵ A more promising prognostic urine biomarker is *SchLAPI*, which has been readily detected in urine sediments through qPCR.²⁵ Since *SchLAPI* was identified as the top overexpressed gene enriched in prostate cancer tissue samples ($n > 1000$) from high-risk patients who eventually experienced metastatic progression versus those who did not,²⁵ *SchLAPI* may better predict for lethal disease than other candidate genes. Preliminary studies suggest that urinary *SchLAPI* expression also exhibits outlier profiles and predicts for more aggressive disease.²⁵ In addition to its potential as a urine biomarker, tissue-based assays for *SchLAPI* are also being developed with *SchLAPI* expression currently available on the clinically used Decipher array,²⁵ and there are also ongoing efforts to validate an RNA *in situ* hybridization assay for *SchLAPI* as well.^{40,77}

In addition to serving as potential prognostic biomarkers (i.e., biomarkers associated with poor outcomes independent of treatment), lncRNAs may also serve as biomarkers which specifically predict response or resistance to particular therapies. The ER α -regulated lncRNA *NEAT1* has been reported to confer resistance to anti-androgen therapies in laboratory models,⁵² additional studies are necessary to determine if this finding validates in clinical samples. More recently, *PARP1* inhibition has been identified as a promising therapeutic approach in patients with castration-resistant prostate cancers harboring alterations in DNA repair genes.⁷⁸ Given preclinical findings that the lncRNA *PCAT1* confers defects in homologous recombination *in vitro* and sensitivity to *PARP1* inhibitors *in vivo*,³⁶ *PCAT1* represents a promising biomarker of response to *PARP1*

inhibition although this finding needs to be further assessed in clinical samples as well.

Ultimately, the “holy grail” in utilizing lncRNAs to personalize therapy will entail the development of successful strategies to target lncRNAs clinically. Currently, RNA interference approaches, with small interfering RNAs (siRNAs), small hairpin RNAs (shRNAs), miRNAs, and antisense oligonucleotides (ASOs), represent a promising strategy for targeting lncRNAs. Within *in vivo* models, targeting *SchLAPI* with shRNA-based approaches decreases metastases in a tail-vein injection model.³⁵ In addition, intratumoral delivery of therapeutic siRNAs directed against *MALAT-1* delays xenograft growth in castrated mice.⁶⁵ ASOs have been developed against *MALAT-1* and demonstrated to be effective in lung cancer xenograft models, supporting the investigation of ASOs in prostate cancer models as well. While RNA interference strategies have shown promise in preclinical models of prostate and other cancers, there are several challenges that must be overcome in the clinical application of these approaches. These issues include optimizing delivery systems for appropriate dosing/distribution and ensuring stability of RNA targeting agents among other issues. To date, a number of siRNA- and ASO-based agents are assessed in both early and late clinical trials for various disease and cancer indications.^{79,80} Further research is necessary to determine if these RNA-targeting strategies can be successfully applied to prostate cancer lncRNAs.

SUMMARY

Recent advances in RNAseq technologies, combined with large-scale efforts to sequence patient samples, have drastically enhanced the discovery of disease-associated lncRNAs.¹⁴ While several prostate cancer lncRNAs promote aggressive phenotypes in preclinical models and are associated with disease progression in clinical cohorts, the underlying mechanisms of these oncogenic lncRNAs need to be further investigated. It is clear that lncRNAs are very promising as diagnostic, prognostic, and predictive biomarkers in prostate cancer. Only time will tell if prostate cancer lncRNAs can be successfully targeted therapeutically, but this area of research holds tremendous potential.

COMPETING FINANCIAL INTEREST

None declared.

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